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Characterization and comparison of poorly known moth communities through DNA barcoding in two Afrotropical environments in Gabon — Source link

Sylvain Delabye, Sylvain Delabye, Rodolphe Rougerie, Sandrine Bayendi ...+14 more authors

Institutions: Sewanee: The University of the South, Academy of Sciences of the Czech Republic, École Normale Supérieure, Laboratory HydroSciences Montpellier

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Complete List of Authors:	Delabye, Sylvain; Faculty of Science, University of South Bohemia in České Budějovice, ; Biology Center, The Czech Academy of Science, Rougerie, Rodolphe; Institut Systématique, Evolution, Biodiversité (ISYEB), Muséum national d'Histoire naturelle, CNRS, Sorbonne Université, EPHE Bayendi, Sandrine; Institut de recherches agronomiques et forestieres, IRAF-CENAREST Andeime-Eyene, Myrianne; Institut de recherches agronomiques et forestieres, IRAF-CENAREST Ayala, Diego; International Centre for Medical Research, CIRMF Zakharov, Evgeny; Biodiversity Institute of Ontario, Centre for Biodiversity Genomics deWaard, Jeremy; Biodiversity Institute of Ontario, Centre for Biodiversity Genomics Hebert, Paul; Biodiversity Institute of Ontario, Centre for Biodiversity Genomics Kamgang, Roger; Laboratoire Evolution, Génomes, Comportement, Ecologie (EGCE UMR 247, IRD–CNRS–Université Paris-Sud) Le-Gall, Philippe; Laboratoire Evolution, Génomes, Comportement, Ecologie (EGCE UMR 247, IRD–CNRS–Université Paris-Sud) Lopez Vaamonde, Carlos; Institut National de la Recherche Agronomique (INRA), ; Institut de Recherche sur la Biologie de l'Insecte (IRBI), Mavoungou, Jacques-François; Institut de Recherches en Ecologie Tropicale (IRET–CENAREST); Faculté des Sciences, Université des Sciences et Techniques de Masuku, Département de Biologie Moulin, Nicolas; Nicolas Moulin Entomologiste Oslisly, Richard; Agence Nationale des Parcs Nationaux (ANPN); Laboratoire Patrimoines Locaux et Gouvernance (PALOC) UMR 208, IRD-MNHN Rahola, Nil; International Centre for Medical Research, CIRMF Sebag, David; Normandie Université, UNIROUEN, UNICAEN, CNRS, M2C UMR 6143 ECOTROP, Team; ECOTROP consortium Decaëns, Thibaud; Centre d'Ecologie Fonctionnelle et Evolutive (CEFE UMR 5175, CNRS–Université de Montpellier–Université Paul-Valéry Montpellier–EPHE),

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Sylvain Delabye[1,2]*, Rodolphe Rougerie[3]*, Sandrine Bayendi[4], Myrianne Andeime-Eyene[4], Diego Ayala[5], Evgeny V. Zakharov[6], Jeremy R. deWaard[6], Paul D.N. Hebert[6], Roger Kamgang[7], Philippe Le Gall[7], Carlos Lopez–Vaamonde[8], Jacques-François Mavoungou[9,10], Ghislain Moussavou[9], Nicolas Moulin[11], Richard Oslisly[12,13], Nil Rahola[5], David Sebag[14], the ECOTROP team[15], Thibaud Decaëns[16]

* Contributed equally to the paper

Addresses:

[1] Faculty of Science, University of South Bohemia in České Budějovice, Branišovská 31, 37005, České Budějovice, Czech Republic

[2] Biology Center, The Czech Academy of Science, Branišovská 31, 37005, České Budějovice, Czech Republic

[3] Institut Systématique Evolution Biodiversité (ISYEB), Muséum national d'Histoire naturelle, CNRS, Sorbonne Université, EPHE, 57 rue Cuvier, CP 50, 75005 Paris, France.

[4] Institut de Recherches Agronomique et Forestière (IRAF–CENAREST), Libreville, Gabon

[5] International Centre for Medical Research (CIRMF), Franceville, Gabon

[6] Centre for Biodiversity Genomics, Biodiversity Institute of Ontario, University of Guelph, Guelph, ON, Canada N1G 2W1

[7] Laboratoire Evolution, Génomes, Comportement, Ecologie (EGCE UMR 247, IRD–CNRS–Université Paris-Sud), Avenue de la Terrasse, Bâtiment 13, Boite Postale 1, 91198 Gif sur Yvette, France

[8] Unité de Zoologie Forestière (URZF UR 633, INRA-Orléans), 2163 Avenue de la Pomme de Pin, CS 40001 Ardon, 45075 Orléans Cedex 2, France

[9] Institut de Recherches en Ecologie Tropicale (IRET–CENAREST), Libreville, Gabon

[10] Département de Biologie, Faculté des Sciences, Université des Sciences et Techniques de Masuku, B.P 943, Franceville, Gabon

[11] Nicolas Moulin Entomologiste, 82 route de l'Ecole, 76680 Montérolier, France

[12] Agence Nationale des Parcs Nationaux (ANPN), Libreville, Gabon

[13] Laboratoire Patrimoines Locaux et Gouvernance (PALOC) UMR 208, IRD-MNHN, 57 rue Cuvier - Case Postale 26, 75231 Paris cedex 05, France

[14] Normandie Université, UNIROUEN, UNICAEN, CNRS, M2C UMR 6143, Place Emile Blondel - Bâtiment IRESE A, 76821 Mont Saint Aignan Cedex, France

[15] A complete list of members of this team, and their affiliations, is given in SI Table 1.

[16] Centre d'Ecologie Fonctionnelle et Evolutive (CEFE UMR 5175, CNRS–Université de Montpellier–Université Paul-Valéry Montpellier–EPHE), 1919 Route de Mende, F-34293 Montpellier, France

Abstract

Biodiversity research in tropical ecosystems – popularized as the most biodiverse habitats on Earth – often neglects invertebrates, yet representing the bulk of local species richness. Insect communities in particular remain strongly impeded by both Linnaean and Wallacean shortfalls, and identifying species often remains a formidable challenge inhibiting the use of these organisms as indicators for ecological and conservation studies.

Here we use DNA barcoding as an alternative to traditional taxonomic approach for characterizing and comparing the diversity of moth communities in two different ecosystems in Gabon. Though sampling remains very incomplete, as evidenced by the high proportion (58%) of species represented by singletons, our results reveal an outstanding diversity. With about 3500 specimens sequenced and representing 1385 BINs (Barcode Index Numbers, used as a proxy to species) in 23 families, the diversity of moths in the two sites sampled is higher than the current number of species listed for the entire country. Both seasonal and spatial turnovers are strikingly high (18.3% of BINs shared between seasons, and 13.3% between sites), emphasizing the need to account for these when running regional surveys. Our results also highlight the richness and singularity of savannah environments and emphasize the status of Central African ecosystems as hotspots of biodiversity.

Keywords: Community Ecology, DNA barcodes, Lepidoptera, Tropical Africa, Taxonomic deficit

Introduction

Tropical ecosystems host unrivalled species richness (Kier *et al*. 2005; Myers 1984; Myers *et al*. 2000), a fact that has long captivated public attention and raised concerns about the way to conserve this immense biodiversity (Wilson 1988). Understanding of tropical biodiversity has historically been biased toward the largest organisms such as angiosperms and vertebrates (May 2011), leaving considerable gaps in our knowledge of hyperdiverse groups of smaller animals, especially arthropods. These organisms are nevertheless key to ecosystem functioning (Erwin 1983; Zhang 2011) and the shortfalls in our taxonomic, biogeographic and ecological knowledge are strong impediments against the integration of these organisms in conservation and management strategies (Miller & Rogo 2002; Whittaker *et al*. 2005). Because the few studies addressing this topic predict high extinction numbers for insects (Fonseca 2009; Stork & Habel 2013), it is urgent to lift "the curse of ignorance" (Diniz-Filho *et al*. 2010) by developing multi-scale studies on insect diversity that benefit from the technological revolution of the 'genomic era' (Godfray 2006; Wilson 2003) and its recent developments in biodiversity sciences (Hebert *et al*. 2003a).

The Afrotropical region is one of the Major Tropical Wilderness Areas on earth (Myers 1990; Wilson 2002), i.e. a large and highly diverse area that has seen little impact from human activities until recently (i.e. < 5 inhab km^{-2} and > 75% of the original vegetation still present) (Mittermeier *et al*. 1998). However, recent estimates indicate that annual net deforestation of African tropical rainforests, although less dramatic than in Latin America or Southeast Asia, approached 0.3 million ha/year for the 2000-2010 decade (Achard *et al*. 2014), which could have led to dramatic biodiversity loss. As many as 100,000 insect species have been reported from the area, but Miller and Rogo (2002) suggest that species richness could exceed 600,000 species. In Gabon, a central-African country which is still covered by 80% of tropical rainforests, insect inventories have only considered butterflies (vande Weghe 2010), a few

groups with limited number of species such as Mantodea (Roy 1973), Lucanidae (Maes & Pauly 1998) or Apoidae (Pauly 1998), and groups with specific economical and/or agronomical importance such as Pseudococcidea (Hemiptera) and their parasitoids (Boussienguet *et al*. 1991). A few studies have also targeted terrestrial arthropod assemblages along human disturbance gradients (Basset *et al*. 2004, 2008).

Several authors emphasized the potential of using highly diverse groups, such as Lepidoptera, as environmental indicators (Axmacher *et al.* 2004a, 2004b; Beck *et al.* 2013; Kitching *et al.* 2000; Ricketts *et al.* 2001). They are indeed key herbivores and an important link within foodwebs as prey or as hosts for parasitoids. Variation in the diversity and structure of Lepidopteran communities is thus likely to be representative of changes at other trophic levels. For instance, lepidopteran species depend on their host plant species (or a few closely related plants), and in turn play a fundamental role as pollinators; this connects them closely to plant community structure and composition (Ehrlich & Raven 1964; Novotny *et al.* 2002b). On the other hand, trophic cascades in food webs are likely to link both host plant and primary consumer assemblages to associated higher trophic levels of predators and parasitoids. Surprisingly however, only a few studies have examined this group in the Afrotropics. The taxonomic deficit and the high number of species that occur in those environments are certainly important causes for this deficit, because they impede reliable inventories and the description of community patterns. In a recent study based on a substantial sampling effort in Papua New Guinea (over 30,000 specimens collected over several years), Ashton *et al.* (2014) found that no asymptote was reached by species accumulation curves. These authors, however, also suggested that more limited sampling could be efficient in highlighting differences in the diversity and composition of moth communities among distant localities.

In this study, we use DNA barcodes to document and compare communities of moths in two differing ecosystems of Gabon. Several recent studies have demonstrated the effectiveness of DNA barcoding – a tool for species identification based on a short standardized DNA fragment (Hebert *et al.* 2003b) – in documenting species diversity of lepidopteran communities in regions where species assemblages are very diverse and when many species are undescribed (Janzen et al., 2009; Lamarre *et al.* 2016; Lees *et al.* 2014; Zenker *et al.* 2016). With this approach we aim at evaluating the sampling effort required to produce relevant census of these communities, to document seasonal variation in community composition, and if species-turnover (β-diversity) as revealed from our data is reflecting significant differences in richness and composition that can be linked to the different habitats sampled. Finally, we discuss the contribution of our study to the current knowledge of moth diversity in Gabon and in the Afrotropical region.

Material and Methods

Study sites

Moths were collected at two locations (named *Lopé 2* and *Ipassa* research station) in the province of Ogooué-Ivindo, in Gabon (Figures 1 and 2):

- *Lopé 2* site is situated in the northern part of Lopé National Park, about 12 km south from Lopé village and the Dr. Alphonse Mackanga Missandzou Training Centre (CEDAMM, Wildlife Conservation Society; coordinates: S0°13'9.699" / E11°35'5.6394"; altitude: 300m). Vegetation comprises a mosaic of forest and shrub savannah (Figure 2A). Shrub savannah is dominated by Poaceae and Cyperaceae like *Anadelphia arrecta*, *Andropogon pseudapricus*, *Schizachyrium platyphyllum*, *Hyparrhenia diplandra* or *Ctenium newtonii* and by a shrub layer with *Crossopteryx febrifuga* and *Nauclea latifolia* (White & Abernethy 1997). Forest patches are mainly secondary to mature okoumé rainforests, the dominant forest type in

western Gabon, dominated by *Aucoumea klaineana ("okoumé")*, *Lophira alata, Desbordesia glaucescens*, *Scyphocephalium ochochoa*, *Dacryodes buttneri*, *Santiria trimera*, *Sindoropsis le-testui* and *Uapaca guineensis* (Ben Yahmed & Pourtier 2004; White & Abernethy 1997).

- The *Ipassa* research station (Institut de Recherches en Ecologie Tropicale) is situated in the northern part of Ivindo National Park, 12 km from the city of Makokou (coordinates: N0°30'38.1456" / E12°48'1.2594"; altitude: 500m). The site is mainly surrounded by mature Guineo-Congolean rainforest showing both Atlantic and continental influences (Doumenge et al. 2004; Nicolas 1977; White 1983), with *Baphia leptobotrys* and *Millettia laurentii* dominating the tree cover, as well as *Scorodophloeus zenkeri*, *Plagiostyles africana*, *Dichostemma glaucescens*, *Santiria trimera*, *Polyalthia suaveolens* and *Poncovia pedicellaris* (Figure 2B).

The two sites are 160 kilometers apart and share a similar seasonal cycle typical of the equatorial transition zone, with short (January-February) and long (June-September) dry seasons. The average monthly temperature is 24°C while mean annual precipitation is 1500 mm at Lopé and 1700 mm at Ipassa.

Moth sampling

Sampling was conducted at both sites in November 2009 and at *Lopé 2* in February-March 2011 during a field class organized in the Lopé National Park (ECOTROP field class - http://www.ecotrop.com/ECOTROP). We used a standard light trap technique consisting of a 250W UV (mercury vapor) bulb placed 4-5 meters above the ground to attract insects (Figures 2A and 2C). Two low voltage lamps (80W) were positioned on both sides of a vertical white sheet positioned below the UV bulb. Specimens were collected during dark-moon phases from dusk to dawn (6pm to 6am, local time) in order to collect species with varying flight times (Lamarre *et al.* 2015). Overall, four collecting nights were carried out in

each site in 2009 (10th to 14th of November at *Lopé 2* and 14th to 18th of November at *Ipassa*), and three additional nights at the end of the short dry season at *Lopé 2* in 2011 (27th February, 1st and 4th March). Our sampling design was therefore relevant to compare observed communities between sites from the samples collected during the rainy season in 2009, and to investigate seasonal turnover at *Lopé 2*.

Our study focuses on Macroheterocera, i.e. macromoths whose wingspan were >1 cm (Figure 2D). Each night and in all families of Macroheterocera, we sampled as many species as could be distinguished morphologically when collecting. Specimens were killed using a cyanide jar or by an injection of ammonia into the thorax for larger species. Moths were placed in glassine envelopes marked with a code unique to each sampling event. Specimens were subsequently sorted into morphospecies, i.e. groups of specimens that were readily distinguishable from their external morphology. A maximum of four specimens per morphospecies and per collecting night were selected for molecular analyses. Specimens are currently deposited in the Museum national d'Histoire Naturelle in Paris, where they are available for further taxonomic study.

DNA barcoding and taxonomic assignments

A small piece of tissue (generally a complete leg or its tarsus for the largest species) was sampled for each specimen selected in the field (Figure 2D). DNA extraction was carried out at the Canadian Centre for DNA Barcoding (CCDB) at the University of Guelph following a standard automated protocol (Ivanova et al. 2006; Hajibabaei *et al.* 2005). Tissue lysis occurred in 50µl of lysis buffer and proteinase K [0.02 mg/µL] incubated at 56ºC overnight. A 658 bp segment of the 5' region of the COI mitochondrial gene used as a standard DNA barcode was amplified through PCR using the primer pair LepF1/LepR1 (Hebert *et al.* 2004). Samples failing to amplify after this first PCR pass were re-processed using the primer sets LepF1/MLepR1 and MLepF1/LepR1 that target 307 bp and 407 bp overlapping fragments,

186 respectively (Hajibabaei *et al.* 2006). A standard PCR reaction protocol was used for all PCR
187 amplifications and products were checked on a 2% E-gel 96 Agarose (Invitrogen). Unpurified
188 PCR amplicons were sequenced in both directions using the same primers as those used for
189 the initial amplification, and following standard CCDB protocols (http://ccdb.ca/resources/)
190 (Hajibabaei *et al.* 2005). Trimming of primers, sequence editing and contig assembly were
191 carried out at CCDB using CodonCode software (CodonCode Corporation, Centerville, MA,
192 USA). All sequences were aligned and inspected for frame-shifts and stop codons for removal
193 of editing errors and possible pseudogenes, and then uploaded in the Barcode of Life Data
194 systems (BOLD, Ratnasingham & Hebert 2007). All records – including specimen and
195 sequence data – can be accessed publicly in BOLD and GenBank, and were assembled within
196 BOLD dataset DS-LOPELEP1.

197 Species assignments of specimens using morphology, either as named species through formal
198 identification or as provisionally delineated morphospecies, could not be achieved for all the
199 specimens, because of the lack of taxonomic expertise for many of the moths collected and
200 because processing the large number of specimens (spreading of wings and often genitalic
201 dissections) was intractable. Because morphospecies are unreliable for a thorough assessment
202 of observed species diversity (Zenker *et al.* 2016), we used DNA barcodes to delineate
203 molecular taxonomic units (MOTUs) as a proxy for species. More specifically, we used
204 Barcode Index Numbers (BINs) derived from the automated MOTU delineation tool
205 implemented in BOLD (Ratnasingham & Hebert 2013), and which have already been used to
206 consistently approximate species in Lepidoptera (Hausmann *et al.* 2013; Kekkonen & Hebert
207 2014). In two families, Saturniidae and Sphingidae, species were carefully identified (by RR
208 and TD) on the basis of morphology and the results were used to test their correspondence
209 with BINs.

For most specimens, we were able to provide a family-level identification based on their general morphology during tissue sampling, or, subsequently using DNA barcode results coupled with the BOLD identification tool as well as the topology of the NJ tree. For this second approach, the richness of the BOLD DNA barcode library, with records for more than 100,000 species of Lepidoptera – proved very useful using a simple query for best close matches in the database. Instead of applying a (necessarily subjective) threshold to generate family (or occasionally subfamily and genus) assignment, we verified the proposed assignments by comparing images and, where relevant, by examining the specimens and confirming the proposed taxon on the basis of its morphology.

Community data analyses

The α-diversity at each site was assessed by plotting rarefaction curves and their extrapolations for both species richness and sample coverage, using specimen numbers as a measure of sampling intensity. These analyses were carried out using the *iNEXT* package (Hsieh *et al.* 2014) for R 3.0.2 (R Development Core Team 2004). We then used the *Vegan* package (Oksanen *et al.* 2013) to calculate several diversity indices: observed richness (defined as the total number of observed BINs at a given sampling site or on a given date), Chao1, ACE and second order jacknife diversity estimators, and Fisher α-diversity index. We also used *iNEXT* to calculate the number of species observed given a constant level of sampling coverage, and *Vegan* for the estimation of species richness rarefied to a constant level of sampling intensity (i.e. a constant number of specimens collected). We finally used *fisherfit*, *prestonfit* and *prestondistr* functions of *Vegan* to plot rank-abundance diagrams and fit Fisher's logseries, Preston's lognormal and truncated lognormal models to abundance data for each sampling site.

To assess β-diversity among sampling sites (for samples collected in 2009) and seasons (in *Lopé 2* site only), we calculated an average Sørensen's index of dissimilarity using the package *Vegan* (Oksanen *et al.* 2013):

$\beta_{BC} = (b+c)/(2\,a + b + c)$

where *a* is the number of species (here BINs) shared between two sites B and C, and *b* and *c* are the numbers of unique BINs for sites B and C.

We used the *betapart* package to decompose β-diversity into two components (Baselga 2010): nestedness (i.e. when the composition of communities with a smaller species number is a subset of a richer community) which reflects non-random processes of species loss, and spatial turnover which results from species replacement as a consequence of environmental sorting or spatial and historical constraints (Qian *et al.* 2005; Ulrich *et al.* 2009; Wright & Reeves 1992). Analyses of β-diversity were carried out with and without singletons (i.e. BINs represented by a single specimen in the dataset), as their inclusion can lead to overestimation of β-diversity.

Results

Species richness at the regional scale

We obtained 3494 (97.7%) sequences from the 3576 specimens selected for DNA barcoding. These sequences included representatives of 1385 BINs representing 23 families of Lepidoptera (Table 1) and only 6 specimens (6 BINs) could not be identified to family level. Noctuidae, Erebidae and Geometridae represented about one third of the BINs and sampled individuals, whereas 10 other families were each represented by less than 10 specimens. More than half of the BINs (786 in total, 57%) were represented by a single individual in our data set (i.e. singleton).

Morphological examination of specimens in the families Saturniidae (177) and Sphingidae (267), led to the distinction of 42 and 63 species, respectively, of which only two (in family Saturniidae) could not be identified to species and were given a provisional name (*Orthogonioptilum* mgab_RR01 and *Dogoia* mgab_RR01). The correspondence between morphologically assigned species and BINs was nearly perfect: 42 species versus 43 BINs in Saturniidae (98%) and 63 versus 66 in Sphingidae (95%) (see DNA barcode NJ trees in SI Figures 1 and 2). In other families, 112 species (representing 121 BINs) were formally identified by taxonomic experts (see acknowledgments) or through DNA barcode matches in BOLD. Overall, with Saturniidae and Sphingidae included, these species represent about 16% of all BINs (230/1385).

Comparison between the number of BINs observed in our study and the list of recognized species and subspecies for Gabon, as derived from the AfroMoths online database (De Prins & De Prins 2017), revealed the strong taxonomic deficit that characterizes moth diversity in the Afrotropics. Afromoths is based on the survey of 7355 published sources for the whole Afro-tropical region (as of August 8th, 2017) and the authors' own studies. It lists 1,301 moth species and subspecies for Gabon, belonging to 36 families. Our survey (Figure 4), limited to macro-moths collected during only 11 nights at two sites, revealed 1385 BINs in just 25 families. Three families (Bombycidae, Brahmaeidae, and Lecithoceridae) detected in our study lack published records for Gabon in the AfroMoths database. For 10 of the 22 other families, the number of BINs recorded in our study exceeded the number of known species (Table 1). Large differences were observed for Cossidae (1 species in AfroMoths versus 11 BINs), Crambidae (9 vs. 52), Erebidae (309 vs. 369), Geometridae (184 vs. 220), Lasiocampidae (68 vs. 101), Noctuidae (71 vs. 224), and Pyralidae (6 vs. 70), which may represent the most understudied families or those yet incompletely surveyed in the AfroMoths database.

281 In the few families that are well-studied for this region, we collected approximately half the
282 known number of species (48.2%, sd=6.9, N=4 – including Saturniidae (43 BINS vs. 110
283 species listed in AfroMoths, 39%), Eupterotidae (15 vs. 32, 47%), Sphingidae (66 vs. 124,
284 53%) and Lasiocampidae (101 vs. 188 as listed by P. Basquin, personal communication,
285 54%).

286 ***Species richness and diversity patterns between sampling sites***

287 Our survey revealed a total of 823 BINs (1604 specimens analyzed) and 782 BINs (1890
288 specimens analyzed) in the *Ipassa* and *Lopé 2* sites, respectively (Table 1). Sampling resulted
289 in a high proportion of singletons at both sites (64% in *Ipassa*, 59% in *Lopé 2*; 57% when
290 combining both sites), and the distributions of BIN abundance are a strong fit to a log-series
291 model (SI Figure 3). While observed richness was similar between the sites, we collected
292 fewer BINs in *Lopé 2*, despite collecting three additional nights in this site during the dry
293 season.

294 For comparison of the two sites, we only considered specimens collected during the wet
295 season when sampling efforts were identical. The four collecting nights at each site resulted in
296 the capture of 1604 and 1110 specimens, which belonged to 823 and 481 BINs for *Ipassa* and
297 *Lopé 2*, respectively (Table 2). Richness estimators indicate that species richness ranged
298 between 1250 and 1850 species at *Ipassa* and between 700 and 1200 species at *Lopé 2*.
299 Rarefaction curves clearly show a higher richness in *Ipassa* (Figure 5a), while sampling
300 coverage rate was slightly higher at *Lopé 2* (73% versus 68% at *Ipassa*) (Table 2, Figures 4b
301 and 4c). Overall, the moth communities at both sites showed a similar relative abundance of
302 the different families, both in terms of specimen numbers and BINs, although observed
303 richness in the most diverse families was consistently higher in *Ipassa*, with the exception of
304 Crambidae and Pyralidae, which had more BINs at *Lopé 2* (Table 1).

Comparison of BINs collected during the wet season at *Lopé 2* and *Ipassa* revealed only 158 BINs shared by the two sites, 13.8% of the total number analyzed. Sørensen's index of β-diversity calculated between the two sites was 0.76 for the whole dataset and 0.42 after singletons were removed (Table 3). In both cases, β-diversity was mainly explained by spatial turnover (71.0% and 67.6%, respectively) and to a lesser extent by nestedness (29.0% and 32.4%).

Seasonal changes in moth assemblages at Lopé 2

We generated DNA barcodes for 1110 and 780 specimens from *Lopé 2* during the rainy and the dry seasons, respectively. Observed richness during the wet season was slightly higher (478 BINs versus 441 BINs during the dry season), but this trend was reversed after rarefying richness to a constant sampling effort or a constant sampling coverage. Rarefaction curves and diversity estimators were also quite similar, the later ranging between 650 and 1100 for both seasons (Figure 5).

During the dry season, we collected moths belonging to 17 families versus 21 families during the wet season. Seven families were not shared between the two sampling seasons, but all were represented by few BINs (maximum 2) and individuals (maximum 2), excepting one BIN in the family Thyrididae for which 18 specimens were collected in the wet season. Overall, the diversity for each family was similar for the two sampling periods (Table 1) with a few exceptions: the Crambidae (31 vs. 16 BINs), Pyralidae (37 vs. 18), and Saturniidae (31 vs. 9), which were all more diverse during the wet season, and the Sphingidae (40 vs. 28) that was more diverse during the dry season. Out of a total of 782 BINs, 144 (i.e. 18.5%) were found during both the rainy and the dry seasons. Sørensen's index of dissimilarity between seasons was 0.69, largely explained by temporal turnover (95.4%), but it dropped to 0.23 and was evenly explained by turnover and nestedness after removing singletons from the data set (Table 3).

Discussion

DNA barcodes for the study of moth diversity in the tropics

Of the 25 moth families identified among more than 3500 collected specimens, three (namely Bombycidae, Brahmaeidae and Lecithoceridae) have no record from Gabon in the AfroMoths database (De Prins & De Prins 2017) and ten have a number of BINs equal or higher than the number of species currently reported therein. Overall, we documented 1385 species-level molecular units (BINs), a number slightly higher than the 1301 species listed for the country in AfroMoths. Considering the relatively shallow geographical range and temporal extent of our study, this result highlights the weakness of the current knowledge of moth diversity in the Afrotropics, despite the remarkable efforts by De Prins & De Prins (2017) to synthesize and centralize this knowledge in the AfroMoths database. Our results clearly highlight the value of DNA barcoding for producing a rapid and accurate census of moth diversity in a poorly studied tropical region. Because this approach facilitates comparisons between sampling campaigns through barcode matches (as exemplified here between sites, but it can also be applied between countries as currently in progress with a similar campaign in Central African Republic), its systematic implementation would represent a powerful mean to address both the Linnean and Wallacean shortfalls (Lomolino 2004), i.e. the inadequacies in taxonomic and distributional knowledge that characterise most invertebrate taxa in poorly studied regions such as the Congo basin (Whittaker *et al*. 2005).

In our study, the large number of BINs without taxonomic assignation at species level (1155 out of 1385) corresponds both to already known species not yet documented in the BOLD libraries and to species that are new to science. The number and proportion of the later remains unclear and further study by expert taxonomists of the specimens collected is needed, as well as continued efforts to populate DNA barcode reference libraries. In addition, the inflation of species numbers in many families may reflect an incomplete census of Gabonese

355 records in past studies, a considerable task initiated in the Afromoths database, but certainly
356 suffering from the absence of recent dedicated efforts to synthesize Lepidopteran diversity
357 data for this country. The bombycid *Amusaron kolga* (Druce, 1887) and brahmaeid
358 *Dactyloceras lucina* (Drury, 1782) for instance represent new records for their respective
359 families in Gabon, but are species known to occur in neighbouring countries of the Congo
360 basin (De Prins & De Prins 2017). In Lasiocampidae the number of species listed in
361 AfroMoths (68) is identical to the number of species reported from an independent literature
362 survey by a specialist of this family on the African continent (P. Basquin, personal
363 communication). Furthermore, this same taxonomic authority (unpublished results) has
364 recorded approximately 188 Gabonese lasiocampid species in natural history collections
365 worldwide, which clearly demonstrates how insufficient the published data are for this family
366 at the regional scale and is consistent with the number of BINs (101) reported in our study.

367 Molecular data can reveal cases of cryptic species, i.e. species that cannot be distinguished
368 from morphological characters, or that present subtle morphological and/or ecological traits
369 previously ascribed to intra-specific variation or thought to be insignificant for species-level
370 recognition (Janzen *et al.* 2009; Janzen *et al.* 2013; Rougerie *et al.* 2014). In our study, such
371 discrepancies between morphologically identified and molecular species were found in four
372 cases within Saturniidae and Sphingidae, corresponding to supposedly morphological well-
373 defined species that appeared to be split into two or three distinct BINs. This is also likely to
374 be the case in more speciose and less studied families such as Erebidae, Geometridae and
375 Noctuidae, leading to an increase of species numbers in these groups compared to available
376 checklists that are only based on morphologically recognized species.

377 ***Moth diversity at Ivindo and Lopé National Parks***

378 Among the 1385 BINs found in our samples, 796 (i.e. 58% of the total) were represented by a
379 single specimen, which is a high singleton proportion compared to the average of 32% found

by Coddington *et al.* (2009) in a review of tropical arthropod studies. There are little or no biological explanations for the high proportion of rare species usually found in tropical insect surveys (Novotný & Basset 2000). Rather, this pattern can be attributed to undersampling of highly diverse communities (Coddington *et al.* 2009), suggesting that caution should be taken when interpreting the observed patterns of community composition and structure. It also suggests that the estimates of species richness derived from our results probably represent a low estimation of the actual diversity of these ecosystems. Both rarefaction curves and sampling coverage indices (Figure 5, Table 2) support this idea, suggesting that at least twice the number of collected species may occur in the study area.

We found only a few studies that assessed moth local richness in tropical rainforest or savannah ecosystems and that can be readily compared with our own results. Ashton *et al.* (2014) sampled 791 to 2795 species and produced Chao1 estimates ranging from 1478 to 3666 among three rainforest locations in Malaysia. In Costa Rica, Janzen *et al.* (2009) published a census of 2349 species using a DNA barcode-based assessment of macro-moth assemblages in the Area de Conservación Guanacaste. On the other hand, Hawes *et al.* (2009) reported 98 species of Arctiinae (Erebidae), 43 of Saturniidae and 5 of Sphingidae in a primary forest area of Brazilian Amazonia, which is well below our findings in the present study. Variations in the number of species observed among studies are however difficult to interpret, because they can both reflect real differences in species diversity, but can also be biased by differences in sampling efforts and/or sampling performed in different seasons. In fact, the moth sampling by Ashton *et al.* (2014) and Hawes *et al.* (2009) was done through 264 and 30 collecting nights per study site, respectively, while the survey of Janzen *et al.* (2009) was conducted over decades and involved additional sampling methods (in particular, the mass rearing of caterpillars). Comparing the results obtained in different studies and with different sampling intensity requires standardization through rarefaction procedure (Gotelli &

Colwell 2001). Applying this approach to the data from Ashton *et al.* (2014) produces a result different from what can be directly deduced from observed richness (Nakamura A., Ashton L.A., Kitching. R.L., personal communication). For instance, species numbers in their Malaysian sites ranged from 290 to 475 after standardization to a constant sampling effort of 1000 individuals, and between 100 to 270 at a constant sampling coverage of 50%, which was lower to what we found in our two study sites (Table 2). This suggests that Central African rainforests may represent an important hotspot for moth diversity.

Variation in moth diversity and composition among study sites

Our analyses of moth assemblages during the rainy season in the rainforest of *Ipassa* and the savannah/forest landscape of *Lopé 2* unveiled significant differences in both species diversity and composition. As expected from differences in vegetation coverage, the observed and estimated richness were both higher in *Ipassa*. Plant diversity is indeed higher in the rainforest landscape of *Ipassa* than in the shrubland savannahs and peaty marshes that dominate the landscapes of the northern part of Lopé National Park (White & Abernethy 1997). In addition, despite presenting a comparable structure, forests at *Ipassa* are more humid and present higher tree diversity when compared with the gallery forests of *Lopé 2*. These features presumably offer a broader diversity of ecological niches in terms of trophic resources and microhabitats, in particular via the important diversity of epiphytes and lianas (Ben Yahmed & Pourtier 2004).

Difference in species assemblage composition among sites was high, with only 13.3% of BINs found in both. This high β-diversity was mainly attributed to spatial turnover, meaning that undersampling may only weakly account for this variation. This is in contrast with other studies that reported relatively low β-diversity of insect herbivores in comparable tropical rainforest habitats (Basset *et al.* 2012; Novotny *et al.* 2007). This also concords with other studies having reported high species turnovers among sites as long as these comprised enough

variability in vegetation types (Beck & Chey 2007; Ødegaard 2006). In fact, contrasted composition of dominant forest tree species among our study sites may have selected for different assemblages of herbivorous species, as leaf-chewing insects are usually specialized on a single genus of host plants (Novotny *et al.* 2002a, 2002b). Similarly, the presence of herbaceous ecosystems and secondary forests at *Lopé 2* may have also driven the presence of specific species assemblages associated with these open habitats. The high diversity of Crambidae and Pyralidae observed at this site compared to *Ipassa* could for instance be linked to species preferences within these groups for herbaceous host-plants (Kitching *et al.* 2000).

Even if additional sampling is necessary to confirm this finding, these preliminary results suggest that landscapes dominated by a savannah-forest patchwork may host substantial levels of herbivore insect diversity with a high compositional specificity at species level compared to typical tropical rainforests. This argues in favor of a better consideration of savannah ecosystems in both global estimates and conservation strategies of insect biodiversity.

Seasonal variation of moth assemblages

At *Lopé 2* we found little difference in species richness of moth assemblages collected during the rainy and the dry seasons. In contrast, BINs compositions clearly differed from one season to the other, with only 18.3% of the BINs collected being observed in both seasons, and this temporal β-diversity being clearly explained by seasonal turnover rather than by nestedness (Table 3). Composition may simply be influenced by the level of vegetation development during the seasonal cycle, which is well known to influence the phenology of lepidopteran species, or by different climatic preferences linked to the feeding and/or reproductive activity of the moths.

From a methodological point of view, these results highlight the importance of standardizing the period of sampling to provide fully comparable results among different localities. They

also suggest the need of sampling different seasons to obtain a reliable inventory of species at a given study site, as the assemblages observed at the rainy season (the usually preferred period for moth collecting) clearly do not provide a representative overview of the actual species composition of the focal community.

Conclusion

Our study highlights the usefulness of utilizing DNA barcodes for performing rapid analyses of taxonomic diversity and composition of moth assemblages in poorly studied areas. It also stresses the need to accelerate biodiversity inventories in those areas that have been insufficiently explored regarding moths and other poorly studied invertebrates. Central Africa clearly is one of those areas and our results represent the first robust assessment of moth diversity in Gabonese forests and savannahs, highlighting a strongly understudied fauna. The material collected and the DNA barcode library released with this study are thus important contributions and we expect that they will serve the development of knowledge on the diversity and distribution of African moths. In general, studies combining molecular data and traditional taxonomic expertise are critically needed to better document invertebrate communities in tropical areas, especially in the regions where anthropogenic pressures are high and where species extinctions remain unaccounted for because species simply remain undocumented.

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References

Achart, F., Beuchle, R., Mayaux, P., Stibig, H.J., Bodart, C., Brink, A., Carboni, S., Desclee, B., Donnay, F., Eva, H.D, Lupi, A., Rasi, R., Seliger, R., and Simonetti, D. 2014. Determination of tropical deforestation rates and related carbon losses from 1990 to 2010. *Global Change Biology* **20**: 2540-2554.

Ashton, L., Barlow, H.S., Nakamura, A., and Kitching, R.L. 2014. Diversity in tropical ecosystems: the species richness and turnover of moths in Malaysian rainforests. Insect Conservation and Diversity **8**(2): 132–142.

Axmacher, J.C., Holtmann, G., Scheuermann, L., Brehm, G., Müller-Hohenstein, K., and Fiedler, K. 2004a. Diversity of geometrid moths (Lepidoptera: Geometridae) along an Afrotropical elevational rainforest transect. Diversity and Distributions **10**: 293-302.

Axmacher, J.C., Tünte, H., Schrumpf, M., Müller-Hohenstein, K., Lyaruu, H.V.M., and Fiedler, K. 2004b. Diverging diversity patterns of vascular plants and geometrid moths during forest regeneration on Mt Kilimanjaro, Tanzania. Journal of Biogeography **31**: 895–904.

501 Baselga, A. 2010. Partitioning the turnover and nestedness components of beta diversity.
502 Global Ecology and Biogeography **19**: 134-143.

503 Basset, Y., Cizek, L., Cuenoud, P., Didham, R.K., Guilhaumon, F., Missa, O., Novotny, V.,
504 Odegaard, F., Roslin, T., Schmidl, J., Tishechkin, A.K., Winchester, N.N., Roubik,
505 D.W., Aberlenc, H.P., Bail, J., Barrios, H., Bridle, J.R., Castano-Meneses, G.,
506 Corbara, B., Curletti, G., da Rocha, W.D., de Bakker, D., Delabie, J.H.C., Dejean, A.,
507 Fagan, L.L., Floren, A., Kitching, R.L., Medianero, E., Miller, S.E., de Oliveira, E.G.,
508 Orivel, J., Pollet, M., Rapp, M., Ribeiro, S.P., Roisin, Y., Schmidt, J.B., Sorensen, L.,
509 and Leponce, M. 2012. Arthropod Diversity in a Tropical Forest. Science **338**(6113):
510 1481-1484. doi: 10.1126/science.1226727.

511 Basset, Y., Mavoungou, J.F., Mikissa, J.B., Missa, O., Miller, S.E., Kitching, R.L., and
512 Alonso, A. 2004. Discriminatory power of different arthropod data sets for the
513 biological monitoring of anthropogenic disturbance in tropical forests. Biodiversity
514 and Conservation **13**: 709-732.

515 Basset, Y., Missa, O., Alonso, A., Miller, S.E., Curletti, G., De Meyer, M., Eardley, C.,
516 Lewis, O.T., Mansell, M.W., Novotny, V., and Wagner, T. 2008. Changes in
517 arthropod assemblages along a wide gradient of disturbance in Gabon. Conservation
518 Biology **22**: 1552-1563.

519 Beck, J., Ballesteros-Mejia, L., Nagel, P., and Kitching, I.J. 2013. Online solutions and the
520 "Wallacean shortfall": what does GBIF contribute to our knowledge of species'
521 ranges? Diversity and Distributions **19**: 1043-1050.

522 Beck, J., and Chey, V.K.J. 2007. Beta-diversity of geometrid moths from northern Borneo:
523 effects of habitat, time and space. Journal of Animal Ecology **76**: 230-237.

524 Ben Yahmed, D., and Pourtier, R. 2004. Atlas du Gabon. Editions J.A., Paris, France.

525 Boussienguet, J., Neuenschwander, P., and Herren, H.R. 1991. Essais de lutte biologique
526 contre la Cochenille du manioc au Gabon: I. — Établissement, dispersion du parasite
527 exotique *Epidinocarsis lopezi* [Hym.: Encyrtidae] et déplacement compétitif des
528 parasites indigènes. Entomophaga **36**: 455-469.

529 Coddington, J.A., Agnarsson, I., Miller, J.A., Kuntner, M., and Hormiga, G. 2009.
530 Undersampling bias: the null hypothesis for singleton species in tropical arthropod
531 surveys. Journal of Animal Ecology **78**: 573-584.

532 De Prins J, De Prins W (2017) *Afromoths, online database of Afrotropical moth species*
533 *(Lepidoptera).* World Wide Web electronic publication (www.afromoths.net)
534 [accessed on Aug. 8th, 2017]

535 Diniz-Filho, J.A.F., de Marco Jr, P., and Hawkins, B.A. 2010. Defying the curse of ignorance:
536 perspectives in insect macroecology and conservation biogeography. Insect
537 Conservation and Diversity **3**: 172-179.

538 Doumenge, C., Issembé, Y., Mertens, B., and Trébuchon, J.-F. 2004. Amélioration de la
539 connaissance et de la cartographie des formations végétales du parc national de
540 l'Ivindo (Gabon). Rapport de mission d'expertise CIFOR/IRET-CENAREST/CIRAD.

541 Ehrlich, P.R., and Raven, P.H. 1964. Butterflies and plants - A study in coevolution.
542 Evolution **18**(4): 586-608.

543 Erwin, T.L. 1983. Tropical forest canopies: the last biotic frontier. Bulletin of the Ecological
544 Society of America **29**: 14-20.

545 Fonseca, C.R. 2009. The silent mass extinction of insect herbivores in biodiversity hotspots.
546 Conservation Biology **23**(6): 1507-1515.

547 Godfray, H.C.J. 2006. To boldly sequence. Trends in Ecology & Evolution **21**: 603–604.

548 Gotelli, N.J., and Colwell, R.K. 2001. Quantifying biodiversity: procedures and pitfalls in the
549 measurement and comparison of species richness. Ecology Letters **4**(4): 379-391. doi:
550 10.1046/j.1461-0248.2001.00230.x.

551 Hajibabaei, M., deWaard, J.R., Ivanova, N.V., Ratnasingham, S., Dooh, R.T., Kirk, S.L.,
552 Mackie, P.M., and Hebert, P.D.N. 2005. Critical factors for assembling a high volume
553 of DNA barcodes. Philosophical Transactions of the Royal Society B: Biological
554 Sciences **360**: 1959-1967.

555 Hajibabaei, M., Janzen, D.H., Burns, J.M., Hallwachs, W., and Hebert, P.D.N. 2006. DNA
556 barcodes distinguish species of tropical Lepidoptera. Proceedings of the National
557 Academy of Sciences of the United States of America **103**: 968-971.

558 Hausmann, A., Godfray, H.C.J., Huemer, P., Mutanen, M., Rougerie, R., van Nieukerken,
559 E.J., Ratnasingham, S., and Hebert, P.D.N. 2013. Genetic patterns in European
560 geometrid moths revealed by the barcode index number (BIN) system. PLoS ONE **8**:
561 e84518.

562 Hawes, J., Motta, C.d.S., Overal, W.L., Barlow, J., Gardner, T.A., and Peres, C.A. 2009.
563 Diversity and composition of Amazonian moths in primary, secondary and plantation
564 forests. Journal of Tropical Ecology **25**: 281-300. doi: 10.1017/s0266467409006038.

565 Hebert, P.D.N., Cywinska, A., Ball, S.L., and deWaard, J.R. 2003a. Biological identifications
566 through DNA barcodes. Proceedings of the Royal Society of London. Series B:
567 Biological Sciences **270**: 313-321.

568 Hebert, P.D.N., Penton, E.H., Burns, J.M., Janzen, D.H., and Hallwachs, W. 2004. Ten
569 species in one: DNA barcoding reveals cryptic species in the neotropical skipper
570 butterfly *Astraptes fulgerator*. Proceedings of the National Academy of Sciences of
571 the United States of America **101**: 14812–14817.

572 Hebert, P.D.N., Ratnasingham, S., and deWaard, J.R. 2003b. Barcoding animal life:
573 cytochrome c oxidase subunit 1 divergences among closely related species.
574 Proceedings of the Royal Society of London. Series B: Biological Sciences **270**: S96–
575 S99.

576 Hebert, P.D.N., Stoeckle, M.Y., Zemlak, T.S., and Francis, C.M. 2004. Identification of birds
577 through DNA barcodes. PLoS Biology **2**: e312.

578 Hsieh, T.C., Ma, K.H., and Chao, A. 2014. iNEXT: An R package for interpolation and
579 extrapolation in measuring species diversity.

580 Ivanova, N.V., deWaard, J.R., and Hebert, P.D.N. 2006. An inexpensive, automation-friendly
581 protocol for recovering high-quality DNA. Molecular Ecology Notes **6**: 998–1002.

582 Janzen, D.H., Hallwachs, W., Blandin, P., Burns, J.M., Cadiou, J.-M., Chacon, I., Dapkey, T.,
583 Deans, A.R., Epstein, M.E., Espinoza, B., Franclemont, J.G., Haber, W.A., Hajibabaei,
584 M., Hall, J.P.W., Hebert, P.D.N., Gauld, I.D., Harvey, D.J., Hausmann, A., Kitching,
585 I.J., Lafontaine, D., Landry, J.-F., Lemaire, C., Miller, J.Y., Miller, J.S., Miller, L.,
586 Miller, S.E., Montero, J., Munroe, E., Green, S.R., Ratnasingham, S., Rawlins, J.E.,
587 Robbins, R.K., Rodriguez, J.J., Rougerie, R., Sharkey, M.J., Smith, M.A., Solis, M.A.,
588 Sullivan, J.B., Thiaucourt, P., Wahl, D.B., Weller, S.J., Whitfield, J.B., Willmott,
589 K.R., Wood, D.M., Woodley, N.E., and Wilson, J.J. 2009. Integration of DNA
590 barcoding into an ongoing inventory of complex tropical biodiversity. Molecular
591 Ecology Resources **9**: 1–26.

592 Janzen, D.H., Hallwachs, W., Harvey, D., K., D., Rougerie, R., Hajibabaei, M., Smith, M.A.,
593 Chacon, I., Espinoza, B., Sullivan, B., Decaëns, T., Herbin, D., Chavarria, L.F.,
594 Franco, R., Cambronero, H., Rios, S., Quesada, F., Pereira, G., Vargas, J., Guadamuz,
595 A., Espinoza, R., Hernandez, J., Rios, L., Cantillano, E., Moraga, R., Moraga, C.,

596 Rios, P., Rios, M., Calero, R., Martinez, D., Briceño, D., Carmona, M., Apu, E.,
597 Aragon, K., Umaña, C., Perez, J., Cordoba, A., Umaña, P., Sihezar, G., Espinoza, O.,
598 Cano, C., Araya, E., Garcia, D., Ramirez, H., Pereira, M., Cortez, J., Pereira, M., and
599 Hebert, P.D.N. 2013. What happens to the traditional taxonomy when a well-known
600 tropical saturniid moth fauna is DNA barcoded? Invertebrate Systematics **26**: 478–
601 505.

602 Kekkonen, M., and Hebert, P.D.N. 2014. DNA barcode-based delineation of putative species:
603 efficient start for taxonomic workflows. Molecular Ecology Resources **4**(4): 706-715.

604 Kier, G., Mutke, J., Dinerstein, E., Ricketts, T.H., Küper, W., Kreft, H., and Barthlott, W.
605 2005. Global patterns of plant diversity and floristic knowledge. Journal of
606 Biogeography **32**: 1107–1116.

607 Kitching, R.l., Orr, A.G., Thalib, L., Mitchell, H., Hopkins, M.S., and Graham, A.W. 2000.
608 Moth assemblages as indicators of environmental quality in remnants of upland
609 Australian rain forest. Journal of Applied Ecology **37**: 284-297.

610 Lamarre, G., Mendoza, I., Rougerie, R., Decaëns, T., Hérault, B., and Bénéluz, F. 2015. Stay
611 out (almost) all night: contrasting responses in flight activity among tropical moth
612 assemblages. Neotropical Entomology **44**: 109-115.

613 Lamarre, G.P.A., Decaëns, T., Rougerie, R., Barbut, J., deWaard, J.R., Hebert, P.D.N.,
614 Herbin, D., Laguerre, M., Thiaucourt, P., and Martins Bonifacio, M. 2016. An
615 integrative taxonomy approach unveils unknown and threatened moth species in
616 Amazonian rainforest fragments. Insect Conservation and Diversity **9**(5): 475-479.
617 doi: 10.1111/icad.12187.

618 Lees, D.C., Kahawara, A.Y., Rougerie, R., Kawakita, A., Bouteleux, O., De Prins, J., and
619 Lopez-Vaamonde, C. 2013. DNA barcoding reveals a largely unknown fauna of

620 Gracillariidae leaf-mining moths in the Neotropics. Molecular Ecology Resources
621 **14**(2): 286–296.

622 Lomolino, M.V. 2004. Conservation biogeography. *In* Frontiers of biogeography: new
623 directions in the geography of nature. *Edited by* M.V. Lomolino and L.R. Heaney.
624 Sinauer Associates, Sunderland, Massachusetts. pp. 293–296.

625 Maes, J.-M., and Pauly, A. 1998. Lucanidae (Coleoptera) du Gabon. Bulletin et Annales de la
626 Société Royale Belge d'Entomologie **134**: 279-285.

627 May, R.M. 2011. Why worry about how many species and their loss? PLoS Biology **9**(8):
628 e1001130.

629 Miller, S.E., and Rogo, L. 2002. Challenges and opportunities in understanding and utilisation
630 of African insect diversity. Cimbebesia **17**: 197-218.

631 Mittermeier, R.A., Myers, N., Thomsen, J.B., Da Fonseca, G.A.B., and Olivieri, S. 1998.
632 Biodiversity hotspots and major tropical wilderness areas: approaches to setting
633 conservation priorities. Conservation Biology **12**: 516-520.

634 Myers, N. 1984. The primary source: tropical forests and our future. W.W. Norton, New
635 York.

636 Myers, N. 1990. The biodiversity challenge: expanded hot-spots analysis. Environmentalist
637 **10**: 243-256.

638 Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B., and Kent, J. 2000.
639 Biodiversity hotspots for conservation priorities. Nature **403**: 853-858.

640 Nicolas, P. 1977. Contribution à l'étude phytogéographique de la forêt du Gabon. Université
641 de Paris I.

642 Novotný, V., and Basset, Y. 2000. Rare species in communities of tropical insect herbivores:
643 pondering the mystery of singletons. Oikos **89**: 564-572.

644 Novotny, V., Basset, Y., Miller, S.E., Drozd, P., and Cizek, L. 2002a. Host specialization of
645 leaf-chewing insects in a New Guinea rainforest. Journal of Animal Ecology **71**: 400-
646 412.

647 Novotny, V., Basset, Y., Miller, S.E., Weiblen, G.D., Bremer, B., Cizek, L., and Drozd, P.
648 2002b. Low host specificity of herbivorous insects in a tropical forest. Nature
649 **416**(6883): 841-844. doi: 10.1038/416841a.

650 Novotny, V., Miller, S.E., Hulcr, J., Drew, R.A.I., Basset, Y., Janda, M., Setliff, G.P.,
651 Darrow, K., Stewart, A.J.A., Auga, J., Isua, B., Molem, K., Manumbor, M., Tamtiai,
652 E., Mogia, M., and Weiblen, G.D. 2007. Low beta diversity of herbivorous insects in
653 tropical forests. Nature **448**: 692-697.

654 Ødegaard, F. 2006. Host specificity, alpha- and beta-diversity of phytophagous beetles in two
655 tropical forests in Panama. Biodiversity Conservation **15**: 83-105.

656 Oksanen, J., Blanchet, F.G., Kindt, R., Oksanen, M.J., and Suggests, M. 2013. Package
657 "vegan." Community ecology package. R package version 2.0-10.

658 Pauly, A. 1998. Hymenoptera Apoidea from Gabon. Annales - Musee Royal de l'Afrique
659 Centrale - Sciences Zoologiques (Belgium) **282**: 1–121.

660 Qian, H., Ricklefs, R.E., and White, P.S. 2005. Beta diversity of angiosperms in temperate
661 floras of eastern Asia and eastern North America. Ecology Letters **8**: 15-22.

662 R Development Core Team. 2004. R: a language and environment for statistical computing. R
663 Foundation for Statistical Computing, Vienna, Austria.

664 Ratnasingham, S., and Hebert, P.D.N. 2007. BOLD: The Barcode of Life Data System
665 (http://www.barcodinglife.org). Molecular Ecology Notes **7**: 355-364.

666 Ratnasingham, S., and Hebert, P.D.N. 2013. A DNA-based registry for all animal species: the
667 barcode index number (BIN) system. PLoS ONE **8**: e66213.

668 Ricketts, T.H., Daily, G.C., Ehrlich, P.R., and Fay, J.P. 2001. Countryside biogeography of
669 moths in a fragmented landscape: biodiversity in native and agricultural habitats.
670 Conservation Biology **15**: 378-388.

671 Rougerie, R., Kitching, I.J., Haxaire, J., Miller, S.E., Hausmann, A., and Hebert, P.D.N. 2014.
672 Australian Sphingidae – DNA Barcodes challenge current species boundaries and
673 distributions. PLoS ONE **9**(7): e101108.

674 Roy, R. 1973. Premier inventaire des mantes du Gabon. Biologia Gabonica **8**: 235–290.

675 Stork, N.E., and Habel, J.C. 2013. Can biodiversity hotspots protect more than tropical forest
676 plants and vertebrates? Journal of Biogeography **41**: 421-428.

677 Ulrich, W., Almeida-Neto, M., and Gotelli, N.J. 2009. A consumer's guide to nestedness
678 analysis. Oikos **118**: 3-17.

679 Vande Weghe, J.P. 2010. Papillons du Gabon. Wildlife Conservation Society, Libreville.

680 White, F. 1983. The vegetation of Africa: a descriptive memoir to accompany the
681 Unesco/AETFAT/UNSO vegetation map of Africa. Unesco/AETFAT/UNSO. pp. 356.

682 White, L.J.T., and Abernethy, K.A. 1997. A guide to the vegetation of the Lopé Reserve,
683 Gabon. Wildlife Conservation Society, New York.

684 Whittaker, R.J., Araújo, M.B., Jepson, P., Ladle, R.J., Watson, J.E.M., and Willis, K.J. 2005a.
685 Conservation biogeography: assessment and prospect. Diversity and Distributions **11**:
686 3–23.

687 Whittaker, R.J., Araujo, M.B., Paul, J., Ladle, R.J., Watson, J.E.M., and Willis, K.J. 2005.
688 Conservation Biogeography: assessment and prospect. Diversity and Distributions
689 **11**(1): 3-23. doi: 10.1111/j.1366-9516.2005.00143.x.

690 Wilson, E.O. 1988. Biodiversity. National Academies of Sciences, Washington DC.

691 Wilson, E.O. 2002. The future of life. Vintage Books, New York.

692 Wilson, E.O. 2003. The encyclopedia of life. Trends in Ecology & Evolution **18**: 77-80.

693 Wright, D.H., and Reeves, J.H. 1992. On the meaning and measurement of nestedness of
694 species assemblages. Oecologia **92**: 416-428.

695 Zenker, M.M., Rougerie, R., Teston, J.A., Laguerre, M., Pie, M.R., and Freitas, A.V.L. 2016.
696 Fast Census of Moth Diversity in the Neotropics: A Comparison of Field-Assigned
697 Morphospecies and DNA Barcoding in Tiger Moths. PloS one **11**(2): e0148423. doi:
698 10.1371/journal.pone.0148423.

699 Zhang, Z.-Q. 2011. Animal biodiversity: an introduction of higher-level classification and
700 survey of taxonomic richness. Zootaxa **3148** : 7-12.

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Tables

Table 1. Number of individuals and number of BINs collected for different families/sub-families of macro-moths at the two study sites and for two seasons at *Lopé 2*, and number of species listed in the AfroMoths online database (grey bars; De Prins & De Prins 2017) for the same families/sub-families (WS= wet season; DS= dry season).

	Ipassa (WS)		Lopé (WS)		Lopé (DS)		Lopé		Total	
	# ind	# BINs	# ind	# BINs	# ind	# BINs	# ind	# BINs	# ind	# BINs
Bombycidae	2	2							2	2
Brahmaeidae	2	1	2	1			2	1	4	1
Cossidae	6	5	5	4	8	4	13	8	19	11
Crambidae	17	14	73	31	19	16	92	43	109	52
Drepanidae	6	4			1	1	1	1	7	5
Erebidae (Arctiinae)	198	71	131	41	75	37	206	65	404	113
Erebidae (Erebinae)	75	38	71	24	47	34	118	46	193	72
Erebidae (Lymantriinae)	220	103	47	32	79	54	126	73	346	164
Other Erebidae	61	33	102	18	22	18	124	32	185	60
Eriocottidae			2	2			2	2	2	2
Eupterotidae	13	10	22	3	5	3	27	5	40	15
Euteliidae			1	1	4	2	5	2	5	2
Geometridae	293	153	130	63	117	62	247	107	540	220
Lasiocampidae	88	55	80	42	74	36	154	61	242	101
Lecithoceridae			1	1	2	1	3	2	3	2
Limacodidae	31	15	20	14	8	7	28	18	59	30
Noctuidae	199	125	103	66	95	69	198	124	397	224
Nolidae			2	2			2	2	2	2
Notodontidae	155	77	72	31	45	27	117	49	272	104
Psychidae	1	1	2	2	6	4	8	4	9	5
Pyralidae	65	29	82	37	25	18	107	49	172	70
Saturniidae	62	32	79	31	36	9	115	33	177	43

Sphingidae	98	44	58	28	111	40	169	47	267	66
Thyrididae	4	4	18	1			18	1	22	5
Tineidae			2	1			2	1	2	1
Tortricidae	5	4			1	1	1	1	6	5
Uraniidae			1	1			1	1	1	1
Zygaenidae	1	1					0		1	1
Not identified	2	2	4	4			4	4	6	6
Total	***1604***	***823***	***1110***	***481***	***780***	***443***	***1890***	***782***	***3494***	***1385***

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709

710 **Table 2.** Summary of macro-moth data sets collected at the two study sites and for two
711 seasons in *Lopé 2* (numbers in parentheses represent the 95% confidence intervals based on a
712 bootstrap method with 200 replications).

	Ipassa (WS)	**Lopé (WS)**	**Lopé (DS)**	**Lopé**
Number of indiduals collected	1604	1110	780	1890
Observed richness	823	481	443	782
Proportion of singletons (%)	63.85	63.61	64.93	59.31
Sampling coverage (%)	67.32 (± 2.95)	72.44 (± 2.27)	63.14 (± 3.02)	75.54 (± 1.77)
Richness at constant sampling coverage of 50%	469.2 (± 13.8)	197.6 (± 7.0)	313.7 (± 12.9)	330.4 (± 7.6)
Richness at constant sampling intensity of 1000 indiv.	599.1 (± 8.9)	449.9 (± 4.4)	511.4 (± 25.6)	521.5 (± 9.5)
Chao1 estimated richness	1837.0 (± 130.6)	1011.6 (± 107.9)	869.5 (± 70.9)	1513.4 (± 96.5)
ACE estimated richness	1849.4 (± 27.6)	1120.6 (± 20.7)	1054.4 (± 22.9)	1629.5 (± 26.2)
First order jackniffe estimated richness	1269.2 (± 286.2)	728.5 (± 169.7)	663.7 (± 166.9)	1211.4 (± 197.0)
Fisher alpha	678.3 (± 36.5)	318.2 (± 21.0)	419.3 (± 32.3)	491.5 (± 25.0)

713

714

715 **Table 3.** Comparison of macro-moth species assemblages between the two study sites and for
716 two seasons in *Lopé 2* showing the Sørensen index of dissimilitude (with singletons removed
717 or not from the dataset) and its partitioning into geographical/seasonal turnover and
718 nestedness.

	Sørensen	Turnover (%)	Nestedness (%)
Ipassa vs Lopé 2	0.75	70.97	29.03
Same without singletons	0.40	67.57	32.43
Wet vs dry season (Lopé 2)	0.69	95.39	4.61
Same without singletons	0.23	54.76	45.24

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720

721 **Figures**

722 **Figure 1.** Location of the study sites; Dark grey areas on the upper right map represent
723 National Parks in Gabon. The *Ipassa* site is located near Ivindo.

724 **Figure 2.** Photos of the two study sites and sampling methods: A) View of the savannah-
725 forest patchwork in Lopé National Park, showing the position of the light trap (*Lopé 2*,
726 November 2009); B) Rainforests at *Ipassa* research station at the edge of the Ivindo river
727 (November 2009); C) Light trapping at *Lopé 2* in March 2011; D) Tissue sampling for DNA
728 barcoding during the ECOTROP field class in March 2011.

729 **Figure 3.** Diversity and composition of the macro-moth sample at the two locations (*Lopé 2*
730 and *Ipassa*): the circular phylogram represents the results of a Neighbor Joining analysis in
731 BOLD of 3,494 COI sequences clustering into 1,385 BINs; barcodes obtained for specimens
732 from *Ipassa* are in green while those from *Lopé 2* are in grey. The pie chart represents the
733 relative contribution (ordered) of the different families and sub-families (for Erebidae) of
734 moths collected in the two sites; numbers within brackets indicate the number of BINs and
735 number of specimens sampled, respectively.

736 **Figure 4.** Comparisons between the numbers of BINs observed in this study for 28 families
737 and sub-families of macro-moths (dashed bars) and the numbers reported from Gabon in the
738 AfroMoths online database (grey bars; De Prins & De Prins 2017).

739 **Figure 5.** Individual-, sample-, and coverage-based rarefaction and extrapolation curves for
740 the two study sites and for two seasons at *Lopé 2* (DS: dry season, WS: wet season): A) Size-
741 based rarefaction/extrapolation curves; B) Sample coverage plotted against the number of
742 individuals; C) Coverage-based rarefaction/extrapolation (rarefaction curves are represented
743 in solid lines, extrapolation curves in dashed lines; shaded areas represent a 95% confidence
744 intervals based on a bootstrap method with 200 replications).

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747 **Supplementary material**

748 **SI Table 1.** List of members of the ECOTROP team and their affiliations.

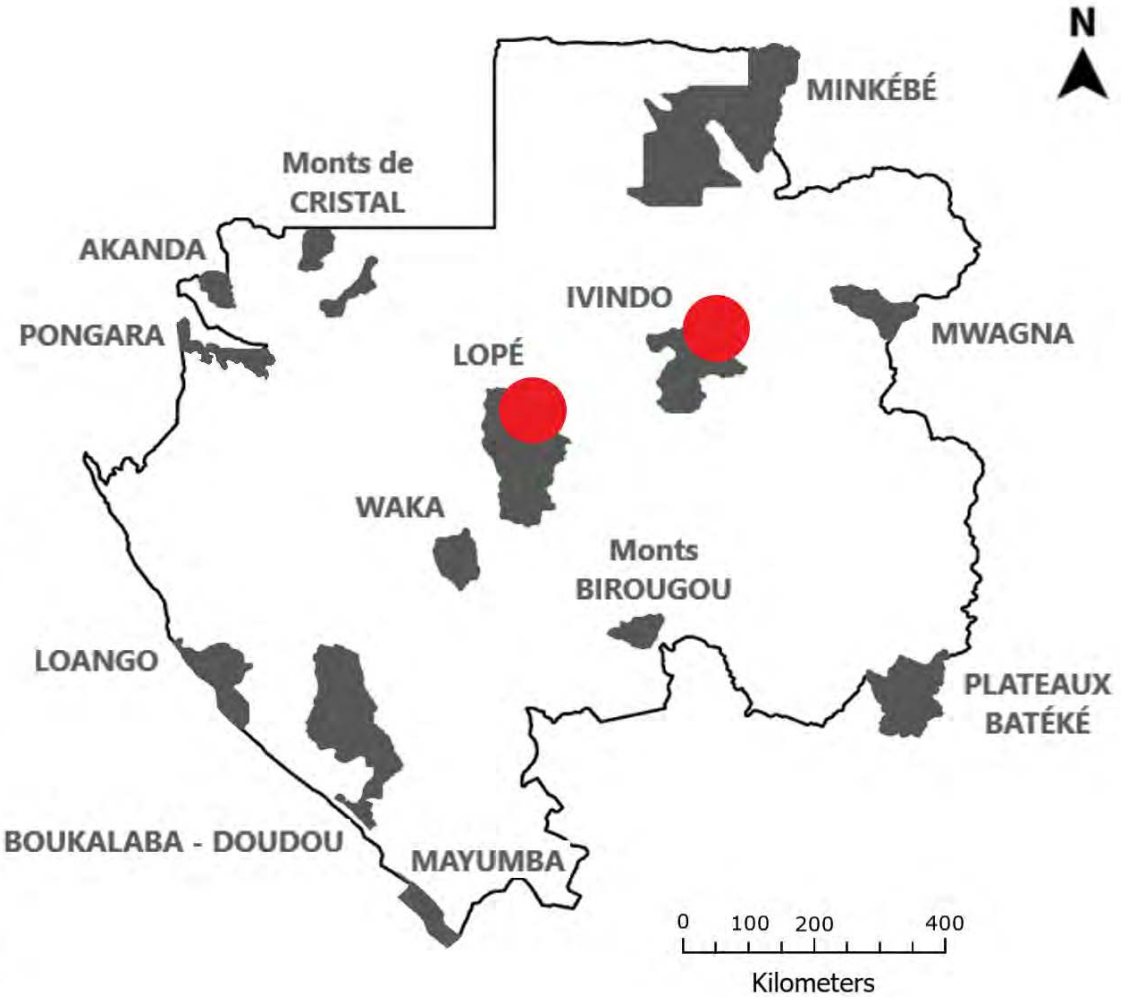
749 **SI Fig. 1.** Neighbour Joining (NJ) tree based on K2P distances for the Sphingidae and
750 Saturniidae moths collected in *Ipassa* (terminals labelled as 'makokou' in the tree) and *Lopé 2*
751 (labelled as 'La Lope'). The tree was produced with records in BOLD dataset DS-LOPELEP1
752 using BOLD-alignment and default settings.

753 **SI Fig. 2.** Images of specimens in NJ tree of SI Fig. 1; numbers of images correspond to
754 numbers of terminals in SI Fig. 1 tree.

755 **SI Fig. 3.** Rank-abundance diagrams for *Ipassa* (left panels) and *Lopé 2* (right panels): (A)
756 and (C) represent Fisher's logseries functions fitted on abundance data; (B) and (D) represent
757 Preston's lognormal (red lines) and truncated lognormal (blue lines) models.

758

759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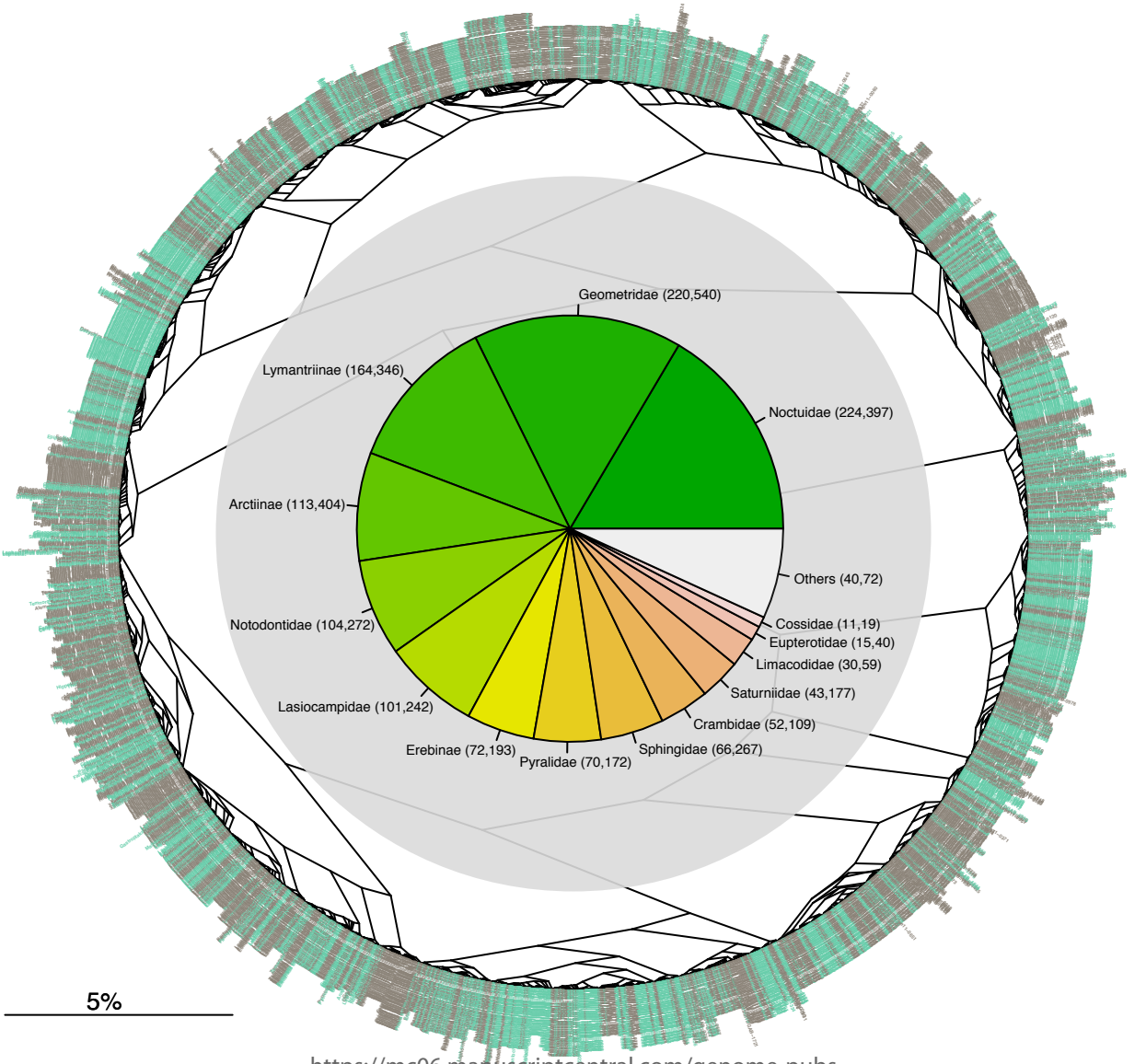
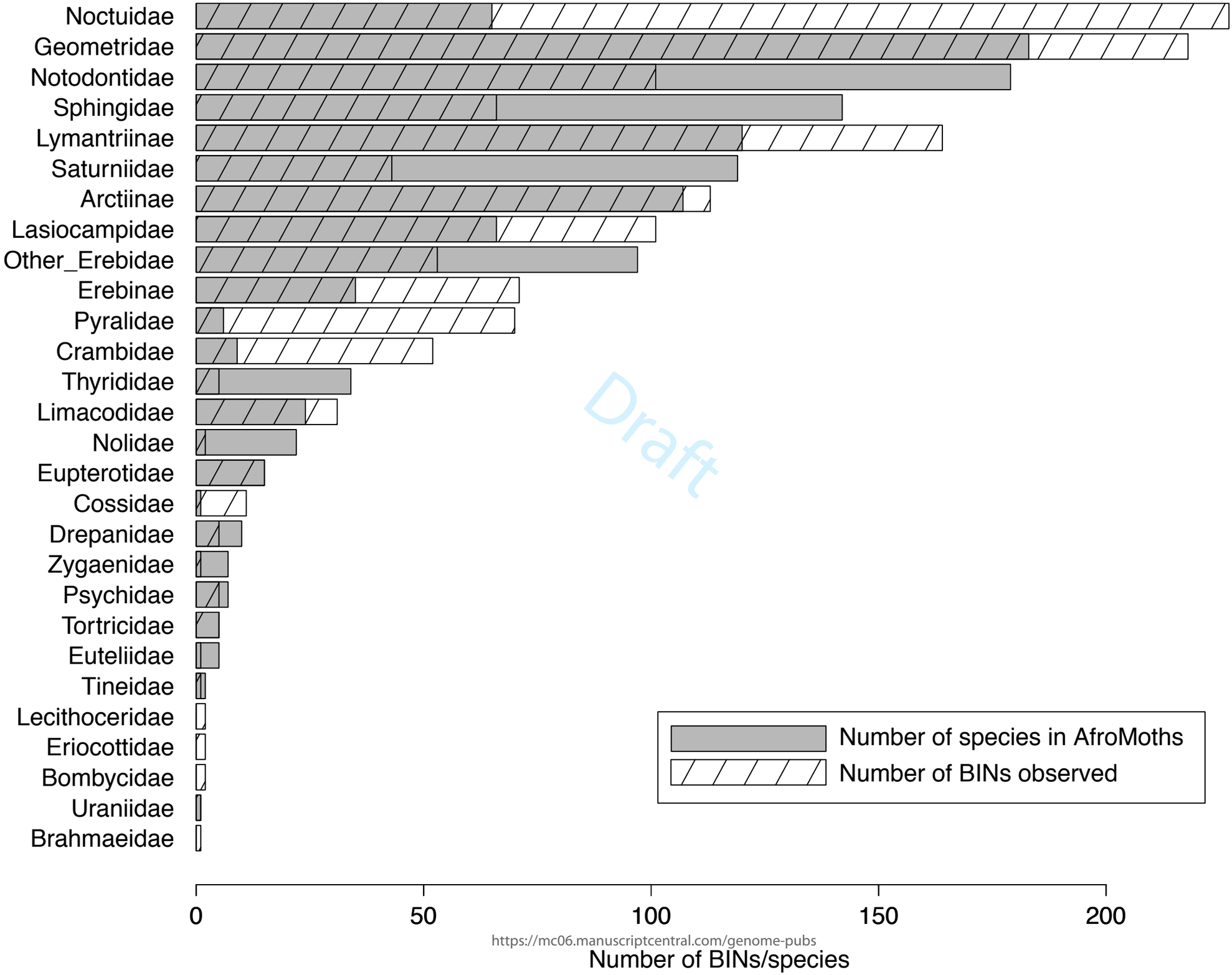
MINKÉBÉ
Monts de
CRISTAL
AKANDA
PONGARA
IVINDO
LOPÉ
MWAGNA
WAKA
Monts
BIROUGOU
LOANGO
PLATEAUX
BATÉKÉ
BOUKALABA - DOUDOU
MAYUMBA
0 100 200 400
Kilometers


Geometridae (220,540)
Noctuidae (224,397)
Others (40,72)
Cossidae (11,19)
Eupterotidae (15,40)
Limacodidae (30,59)
Saturniidae (43,177)
Crambidae (52,109)
Sphingidae (66,267)
Pyralidae (70,172)
Erebinae (72,193)
Lasiocampidae (101,242)
Notodontidae (104,272)
Arctiinae (113,404)
Lymantriinae (164,346)
5%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Geometridae
Notodontidae
Sphingidae
Lymantriinae
Saturniidae
Arctiinae
Lasiocampidae
Other_Erebidae
Erebinae
Pyralidae
Crambidae
Thyrididae
Limacodidae
Nolidae
Eupterotidae
Cossidae
Drepanidae
Zygaenidae
Psychidae
Tortricidae
Euteliidae
Tineidae
Lecithoceridae
Eriocottidae
Bombycidae
Uraniidae
Brahmaeidae
Number of species in AfroMoths
Number of BINs observed
0
50
100
150
200
Number of BINs/species